

Two new haemogregarines, *Haemogregarina waltirensis* n. sp. from *Calotes versicolor* (Daudin) and *H. ganapatii* n. sp. from *Lissemys punctata granosa* (Shoepff)*

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Abstract. Two new haemogregarines, *Haemogregarina waltirensis* n. sp. from *Calotes versicolor* (Daudin) and *H. ganapatii* n. sp. from *Lissemys punctata granosa* (Shoepff) are described. Both occur in the circulating erythrocytes as well as internal organs. The intra-erythrocytic form of *H. waltirensis* n. sp. reach a maximum size of $14.5 \times 5.0 \mu\text{m}$ and was sausage-shaped. The early trophozoites found in blood as well as internal organs contain pink stained granules at the poles. Capsule is absent. Schizogony occurs in liver, lungs and bone marrow. The largest schizont measuring $14.0 \times 11.0 \mu\text{m}$ was found in liver. The gametocytes of *H. ganapatii* n. sp. which are intra-erythrocytic reach a maximum size of $11.5 \times 4.5 \mu\text{m}$ and become sausage-shaped. Capsule is absent. Schizogonic stages are found inside erythrocytes as well as in lungs and liver. The largest schizont in lung measured $11.0 \times 9.5 \mu\text{m}$ while that in the liver measured $7.0 \times 6.5 \mu\text{m}$ both of which contain 24 nuclei.

Keywords. Haemogregarines ; *Haemogregarina waltirensis* n. sp. ; *Haemogregarina ganapatii* n. sp. ; *Calotes versicolor* ; *Lissemys punctata granosa*.

1. Introduction

Several species belonging to the genus *Haemogregarina* Danilewsky, 1885 were reported from vertebrates especially reptiles. In the present study two more species, *H. waltirensis* n. sp. from *Calotes versicolor* (Daudin) and *H. ganapatii* n. sp. from *Lissemys punctata granosa* (Shoepff) are described.

2. Materials and methods

The lizards and turtles were bought from the local vendors. Thin smears were prepared from a drop of blood obtained by clipping the toes or cutting the tail tip, fixed in acetone-free methyl alcohol, stained with Giemsa and examined for protozoan parasites. Some smears were wet-fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin. Internal organs of the infected hosts were fixed in alcoholic Bouin's fluid, sectioned at $8 \mu\text{m}$ thickness, stained with haemato-

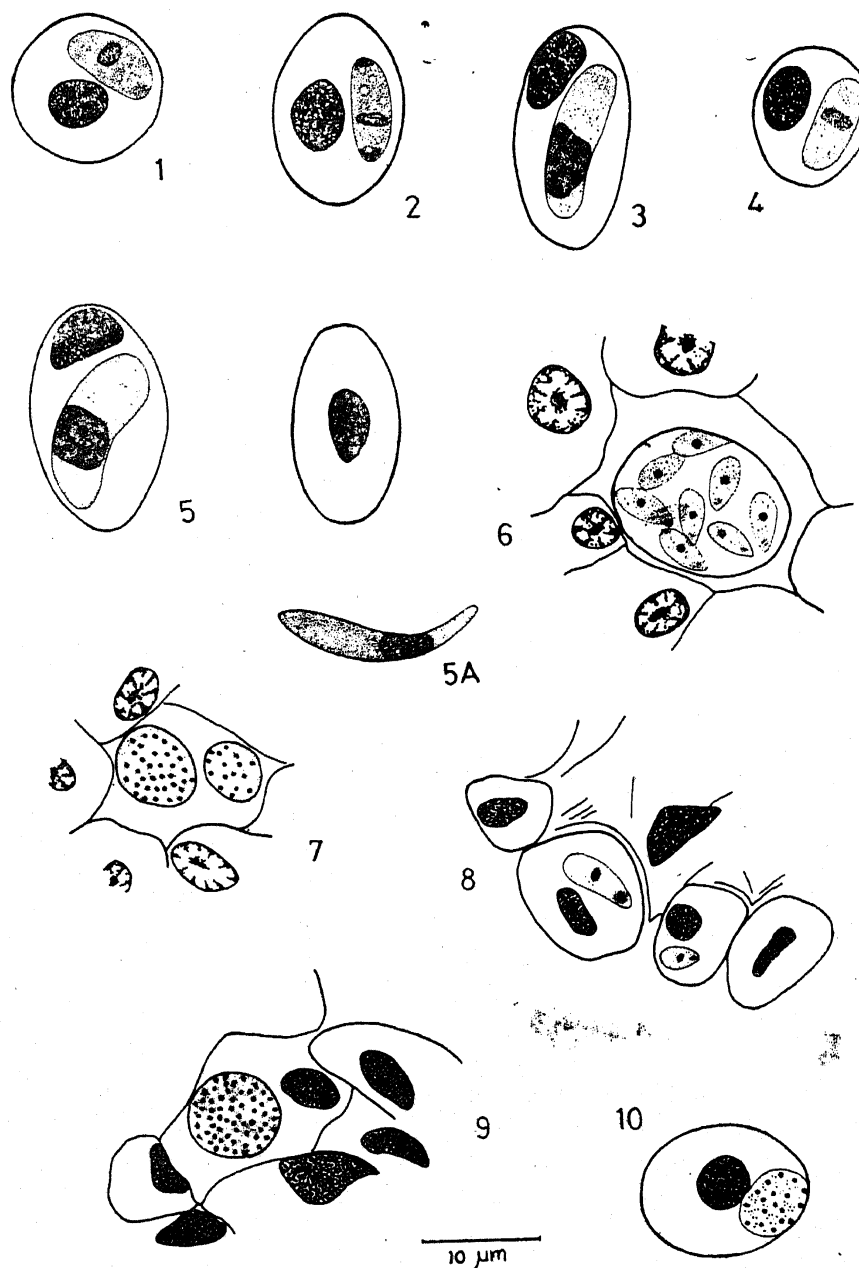
* Based on the Ph.D. Thesis of the author.

xylin and examined for the developmental stages of the parasites. Smears of bone marrow were also prepared, fixed and stained as usual. Figures were drawn with the aid of a camera lucida and measurements were given in microns (μm).

Haemogregarina waltirensis n. sp.

3. Observations

8 out of 140 garden lizards examined were infected with the parasite. Developmental stages of the parasite were found in the peripheral blood as well as internal organs. The smallest parasite observed was sausage shaped measuring $8.0 \times 4.0 \mu\text{m}$ (figure 1). The cytoplasm was vacuolated and stained light blue with



Figures 1-10. *Haemogregarina waltirensis* n. sp. 1-5. Erythrocytic stages. 5A. Free form in plasma. 6, 7. Schizonts in liver. 8. Trophozoites in lung cells. 9. Schizont in the lung cell. 10. Schizont in the bone marrow.

Giemsa. Pink-stained granules were aggregated at one of the poles; in some however, the granules were present at both the poles (figure 2). The nucleus measured $2.5 \times 1.6 \mu\text{m}$ and contained irregularly-stained chromatin granules. A nuclear membrane was not clearly seen. Later stages of the parasite were cigar-shaped, one end being broader containing vacuolated cytoplasm and the other narrow and containing non-vacuolated cytoplasm (figure 3). Some of the parasites while inside the erythrocyte did not show any vacuoles in the cytoplasm (figures 4 and 5). The largest parasite measured $14.5 \times 5.0 \mu\text{m}$ and its nucleus measured $4.8 \times 4.5 \mu\text{m}$. Some of the parasites which were released into the plasma measured $17 \times 3 \mu\text{m}$ (figure 5A).

Schizogonic stages were found in the liver, lungs and bone marrow. The schizonts in the liver appeared intercellular. It may possibly be a result of the disintegration of the host cell as supposed by Ball (1958). The largest schizont measuring $14 \times 11 \mu\text{m}$ contained 9 fully formed merozoites (figure 6). Smaller schizonts showing a greater number of nuclei were also found (figure 7). Apparently there is no relationship between the site of the schizont and the number of nuclei. The merozoites measuring $5-6 \times 2-2.5 \mu\text{m}$ were cigar-shaped, one end being blunt and rounded and the other being narrow and pointed. The cytoplasm was hyaline and contained deeply stained granules at the narrow end. The nucleus was deeply stained and was situated apparently in the centre. The smallest parasite encountered within the lung cells was similar to the merozoites found in the liver and measured $3 \times 1.6 \mu\text{m}$ (figure 8). The largest schizont seen in the lung tissue measured $8 \times 7 \mu\text{m}$ and contained about 50-60 nuclear bits (figure 9). Because of the small size of the schizonts filled with bits of nuclear material it was difficult to ascertain if the merozoites were formed or not. The largest schizont found in bone marrow cells probably immature erythrocytes measured $6.5 \times 4.5 \mu\text{m}$ and contained 18 nuclear bits (figure 10).

When the parasite inside the cell was small it did not have any significant effect on the cell, but with the growth of the parasite, the host cell showed hypertrophy and the nucleus was pushed to a side.

4. Discussion

Seven species belonging to the genus *Haemogregarina* Danilewsky, 1885 were reported from the agamid lizards. In *H. thomsoni* Minchin, 1907, *H. percomsi* Shortt, 1921-22 and *H. cheissini* Ovezmukhammadov, 1974 the cytoplasmic granules were absent unlike in the present form. The nucleus in *H. thomsoni* and *H. percomsi* is near the broad end while in the present case it is at the narrow end. The schizonts of *H. percomsi* are much larger than those of the present form. *H. agamae* Laveran and Pettit, 1909 attacks both erythrocytes and leucocytes. Double infection of the erythrocyte is common in *H. turcomanica* Chodukin and Sofieff, 1940 (cited from Zakharyan 1970) and the unnamed haemogregarine reported from *Agama tuberculata* by Shortt (1916-17) while such phenomenon is not seen in the present form. The capsule which is present in *H. jakimovi* Chodukin and Sofieff, 1940 (cited from Zakharyan 1970), *H. cheissini*, *H. palmeri* Mackerras, 1961 and the unnamed haemogregarine reported by Shortt (1916-17) is absent in the present form. *H. cheissini* is much larger than the present form and causes remark-

able hypertrophy and elongation of the host cell. In the case of the unnamed haemogregarine reported from *Agama rupelli* by Ball (1967) also a capsule is absent as in the present form, but it is much smaller and the granules were distributed along the margin of the parasite. It also contains a large vacuole at one pole in some stages. The present form also differs considerably from *H. triedrii* Robertson, 1908 and *H. leschenaulti* Robertson, 1908 from Ceylon and *H. rodriquesi* de Mello *et al* 1917 and the unnamed species of *Haemogregarina* described by Simond (1901) from *Varanus monitor* from India. For these reasons the present form is considered new and the name, *Haemogregarina waltirensis* n. sp. is proposed for the same.

Species:	<i>Haemogregarina waltirensis</i> n. sp.
Host:	<i>Calotes versicolor</i>
Locality:	Waltair, Andhra Pradesh, India
Site of infection:	Blood, liver, lungs and bone marrow.

Type slides are deposited in the Department of Zoology, Andhra University, Waltair, India.

H. ganapatii n. sp.

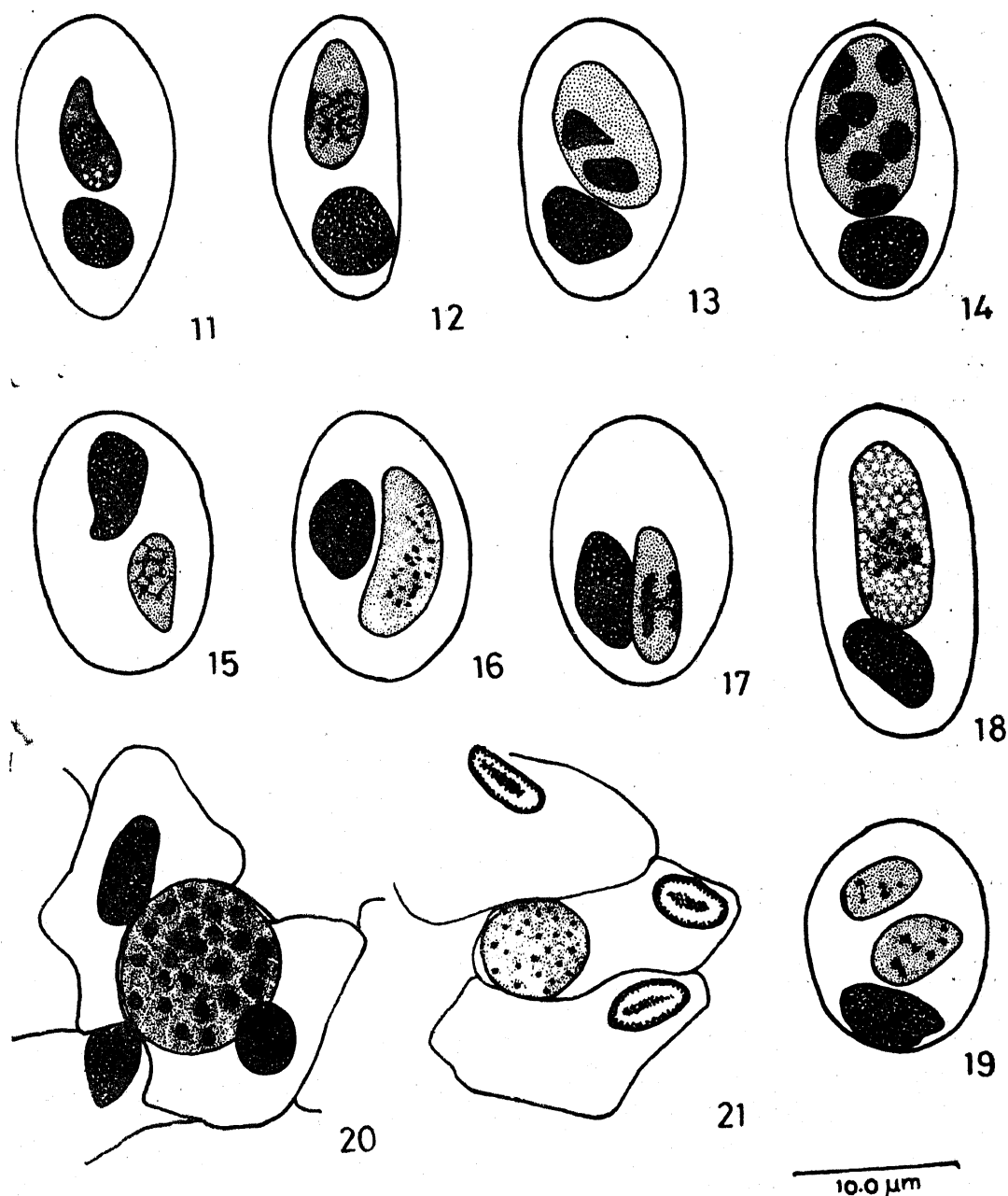
5. Observations

Only 2 out of the 10 terrapins, *L. punctata granosa* harboured the parasite which was found in the erythrocytes as well as lungs and liver. Double invasion of the erythrocytes was rare (figure 19). Erythrocytic schizonts were scarce.

The smallest trophozoite observed in the erythrocyte was pear shaped measuring $7 \times 3 \mu\text{m}$ (figure 11). The cytoplasm which stained light blue with Giemsa contained a few vacuoles. Cell membrane was thin. Capsule was absent. The nucleus was round and the chromatin material was in the form of a close network without any limiting membrane. Parasites measuring $8 \times 3.8 \mu\text{m}$ showed first nuclear division (figure 12). The two nucleate schizont measured $9.5 \times 5.5 \mu\text{m}$ (figure 13). The largest erythrocytic schizont measured $11.5 \times 6.5 \mu\text{m}$ and contained 7 nuclei (figure 14). Unfortunately further stages of erythrocytic schizogony were not found.

The smallest gametocyte observed measured $6 \times 3 \mu\text{m}$ (figure 15). It was similar to the trophozoite except that the cytoplasm was non-vacuolated and the nucleus was a much diffused network of chromatin material occupying about 2/3 of the cytoplasm. In larger forms the nucleus was in the form of pink granules diffused in the cytoplasm and such forms grow up to $10.5 \times 4 \mu\text{m}$ (figure 16). In some cases the chromatin granules were concentrated at the edges (figure 17). Fully grown forms were sausage-shaped measuring $11.5 \times 4.5 \mu\text{m}$ and have finely-vacuolated cytoplasm (figure 18).

Lung was the chief site of exoerythrocytic schizogony and the schizonts reached a maximum size of $11 \times 9.5 \mu\text{m}$ and contained 24 nuclei (figure 20). Very few schizonts were found in the liver. The largest measured $7 \times 6.5 \mu\text{m}$ and contained 24 nuclei (figure 21).



Figures 11-21. *Haemogregarina ganapatii* n. sp. 11-19. Exoerythrocytic stages. 11. Early trophozoite. 12. Form showing nuclear division. 13, 14. Schizonts. 15-18. Gametocytes. 19. Erythrocyte showing double infection. 20. Schizont in lung. 21. Schizont in liver.

6. Discussion

Though several species of *Haemogregarina* were reported from turtles, only 11 species were from India. The unnamed haemogregarine reported by Simond (1901) from *Chitra indica* resembles *H. stepanowi* Danilewsky, 1885 which has cross bands in the gametocyte while the present species lacks them. *H. laverani* Simond, 1901 contains two refringent granules which are absent in the present

form. *H. laverani* and *H. mesnili* Simond, 1901 are amoeboid unlike the present form. The older stages of *H. mesnili* are vermicular with horn-like prolongations bent on itself or oval or reniform or slender and doubled. *H. rara* Laveran and Mesnil, 1902 has its length 5-7 times of its width and has a long nucleus. The younger stages of *H. stepanowiana* Laveran and Mesnil, 1902 are sausage-shaped while its older forms are elongate with the narrow end bent on itself. *H. nicroiae* Castellani and Willey, 1904 is gregarine like with one end granular and the other clear unlike the present form. *H. vittatae* Robertson, 1908 has dense reticulate cytoplasm with two red staining bodies which are perhaps of the nature of plastids and one of the parasites is recurved. *H. testudinis* Laveran and Nattan-Larrier, 1912 have small, medium and large intra-corpuscular forms, free forms and macro- and micro-schizonts containing 4-8 and 12-24 merozoites respectively (cited from Bhatia 1938) and hence differ from the present form. *H. malabarica* de Mello, 1932 has a caudal appendage applied to its concave border. *H. xavieri* de Mello, 1932 has a tail-like recurved projection in the elongate form while the broad forms have two large vacuoles. The gametocytes of *H. gangetica* (Misra *et al* 1974) Misra, 1976 show sexual dimorphism and are much smaller with a compact nucleus unlike the present form. For these reasons the present form is considered new and the name, *Haemogregarina ganapatii* n. sp. in honour of Prof. P. N. Ganapati is proposed for the same.

Species: *Haemogregarina ganapatii* n. sp.

Host: *Lissemys punctata granosa*

Locality: Waltair, Andhra Pradesh, India.

Site of infection: Red blood cells, liver and lungs.

Type slides are deposited in the Zoology Department, Andhra University, Waltair 530 003, India.

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References

- Ball G H 1958 A haemogregarine from a water snake, *Natrix piscator* taken in the vicinity of Bombay, India; *J. Protozool.* 5 274-281
- Ball G H 1967 Some blood sporozoans from East African reptiles; *J. Protozool.* 14 198-210
- Bhatia B L 1938 *The Fauna of British India* (London: Taylor and Francis) pp. 497
- Castellani A and Willey A 1904 Observations on the haematozoa of vertebrates in Ceylon; *Spolia Zeylanica* 2 78-98
- Danilewsky B 1885 Die hematozoen der kaltbluter; *Arch. Mikr. Anat.* 24 588-598
- Laveran A and Mesnil F 1902 Sur quelques protozoaires parasites d'une Tortue d'Asie (*Damonie reevesii*); *C.R. Acad. Sci. Paris* 135 609-614
- Laveran A and Nattan-Larrier L 1912 Contributions al etude de la espundia; *Bull. Soc. Pathol. Exot.* 5 176
- Laveran A and Pettit A 1909 Contribution à l'étude des hémogregarines de quelques sauriens d'Afrique; *Bull. Soc. Pathol. Exot.* 2 506-514

- Mackerras M J 1961 The haematozoa of Australian reptiles ; *Aust. J. Zool.* 9 61-122
- de Mello I F 1932 Contribution á l'étude des hémogregarinides des tortus indiennes ; *Arch. Med. Cirurag. Nova Goa (Ser. A)* 8 1411-1431
- de Mello I F, Bráz de Sá, de Sousa, Loreto, A Dias and Nornha R 1917 Hématozoaires et pseudohématozoaires de l'Inde Portugaise ; *Ann. Sci. Faculd. Med. Porto* 3 5-24
- Minchin E A 1907 On a haemogregarine from the blood of a Himalayan lizard (*Agama tuberculata*) ; *Proc. Zool. Soc. London* 1907 1098-1104
- Misra K K 1976 *Haemogregarina gangetica* a new name for *Haemogregarina simondi* of a river turtle *Trionyx gangeticus* Cuvier ; *Acta Protozool.* 15 21-22
- Misra K K, Nandi N C, Raut S and Chaudhury A 1974 *Haemogregarina simondi* n. sp. a new haemogregarine from a river turtle, *Trionyx gangeticus* Cuvier ; *Acta Protozool.* 12 345-350
- Ovezmukhammadov A 1974 New *Haemogregarina cheissini* sp. nov. recorded on *Agama caucasica* Eichwald ; *Izv. Akad. Nauk. Turkm. SSR Ser. Biol. Nauk.* 6 81-82
- Robertson M 1908 A preliminary note on haematozoa from Ceylon reptiles ; *Spolia zeylanica* 5 178-185
- Shortt H E 1916-17 Notes on two haemogregarines of cold blooded vertebrates ; *Indian J. Med. Res.* 4 402-413
- Short H E 1921-1922 Notes on two haemogregarines of Persian lizards ; *Indian J. Med. Res.* 9 827-829
- Simond P L 1901 Contribution al'étude des hematozoaires endoglobulaires des reptiles ; *Ann. Inst. Pasteur, Paris* 15 319-351
- Zakharyan V Z 1970 Parasitic Protists of reptiles of Fergana Valley ; *Zool. Zh.* 49 298-299

